Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration

Mitsiadis, Thimios A ; Woloszyk, Anna ; Jiménez-Rojo, Lucia

Abstract: Regenerative dentistry represents an attractive multidisciplinary therapeutic approach that complements traditional restorative/surgery techniques and benefits from recent advances in stem cell biology, molecular biology, genomics and proteomics. Materials science is important in such advances to move regenerative dentistry from the laboratory to the clinic. The design of novel nanostructured materials, such as biomimetic matrices and scaffolds for controlling cell fate and differentiation, and nanoparticles for diagnostics, imaging and targeted treatment, is needed. The combination of nanotechnology, which allows the creation of sophisticated materials with exquisite fine structural detail, and stem cell biology turns out to be increasingly useful in regenerative medicine. The administration to patients of dynamic biological agents comprising stem cells, bioactive scaffolds and/or nanoparticles will certainly increase the regenerative impact of dental pathological tissues. This overview briefly describes some of the actual benefits and future possibilities of nanomaterials in the emerging field of stem cell-based regenerative dentistry.

DOI: https://doi.org/10.2217/nnm.12.146

Originally published at:
Mitsiadis, Thimios A; Woloszyk, Anna; Jiménez-Rojo, Lucia (2012). Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration. Nanomedicine, 7(11):1743-1753.
DOI: https://doi.org/10.2217/nnm.12.146

Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration

Thimios A. Mitsiadis*, Anna Woloszyk and Lucia Jiménez-Rojo

Institute of Oral Biology, ZZM, Faculty of Medicine, University of Zurich, 8032 Zurich, Switzerland.

Key words: Nanotechnology, dentistry, stem cells, regenerative medicine, scaffolds, nanomaterials, tooth, odontoblast, dentin, periodontium

Running title: Nanodentistry

*Correspondence: Prof. Dr. Thimios Mitsiadis

University of Zurich, ZZM, Faculty of Medicine

Institute of Oral Biology, Plattenstrasse 11

8032 Zurich, Switzerland.

Email: thimios.mitsiadis@zzm.uzh.ch

Tel: +41 44 634 33 90

Fax: +41 44 634 43 10

Summary

Regenerative dentistry represents an attractive multidisciplinary therapeutic approach that complements traditional restorative/surgery techniques and benefits from recent advances in stem cell biology, molecular biology, genomics and proteomics. Materials science is important in such advances to move regenerative dentistry from the laboratory to the clinic. The design of novel nanostructured materials such as biomimetic matrices and scaffolds for controlling cell fate and differentiation, and nanoparticles for diagnostics, imaging and targeted treatment is needed. The combination of nanotechnology, which allows the creation of sophisticated materials with exquisite fine structural detail, and stem cell biology turn out to be increasingly useful in regenerative medicine. The administration to patients of dynamic biological agents composed by stem cells, bioactive scaffolds and/or nanoparticles will certainly increase the regenerative impact of dental pathological tissues. This overview briefly describes some of the actual benefits and future possibilities of nanomaterials in the emerging field of stem cell-based regenerative dentistry.

1. Pathology and natural regenerative potential of dental tissues

Two of the hardest tissues of the body, the enamel and dentin, form as the outcome of sequential and reciprocal interactions between cells of the oral epithelium and the cranial neural crest-derived mesenchyme [1]. Mesenchymal cells give rise to the dental follicle and dental pulp, while the

oral epithelium forms the ameloblasts. A part of the dental pulp cells differentiate into odontoblasts that produce dentin matrix, whilst ameloblasts form the enamel. When the mineralization of the tooth-crown is completed, the tooth-root starts to develop and subsequently the tooth erupts in the oral cavity. Once root development and cementum deposition have been accomplished the tooth anchors to the surrounding alveolar bone through the periodontal ligament (PDL), which contains extracellular matrix and a great variety of cells such as fibroblasts, epithelial rests of Malassez, endothelial cells.

The mineralized dental tissues are vulnerable to various external harmful agents such as bacteria and acids, but also to traumatic injuries that jeopardize tooth integrity. Although the mitotic and secretory activities of dental pulp and periodontal cells are reduced in adult teeth, these biological processes can be reactivated in pathological conditions (*e.g.*, periodontal and carious diseases) or following traumatic injury [2]. After a mild lesion such as early caries, surviving post-mitotic odontoblasts can produce new dentin through a process known as reactionary dentinogenesis. However, a severe dental injury leads to odontoblast apoptosis that activates dental pulp stem cells to differentiate into new odontoblasts, which are producing the reparative dentin [2]. Periodontal regeneration is a complex process that involves the interaction of several populations of cells that control the specific extracellular matrix components. Cell-occlusive barriers ranging from cellulose to synthetic absorbable materials, which restrict the repopulation of the periodontium from epithelial cells and favor growth of

PDL cells and cementoblasts, are commonly used for periodontium regeneration in dental clinics. These materials are often used in conjunction with biological factors to enhance the regeneration of the alveolar bone. Damaged enamel cannot be repaired naturally since ameloblasts are not present anymore in humans after tooth eruption. Thus, a common practice in dental clinics is to substitute the damaged enamel with biomaterials, ceramics and precious metals.

It is evident that the natural regenerative capacity of dental tissues is often insufficient to entirely restore damaged teeth. In such cases, stem cell biology combined with tissue engineering technology could be useful for the development of innovative strategies for cell-based dental tissue regeneration [3, 4].

2. Stem cell populations within dental and periodontal tissues

Stem cells are undifferentiated cells characterized by their ability to self-replicate throughout life and their capacity to differentiate into diverse specialized cell types [5]. Adult stem cells are found in various tissues of the human body from both epithelial and mesenchymal origin, including skin [6], adipose tissue [7], periosteum [8] and cartilage [9]. Due to their ability to give rise to every cell type in a given tissue, adult stem cells are responsible for tissue/organ homeostasis and regeneration.

Mesenchymal stem cells (MSCs) have been isolated from different locations within adult or postnatal dental tissues. Dental mesenchymal stem cells (DMSCs) have been isolated from the pulp of adult and deciduous teeth (DPSCs and SHEDs respectively) [10], apical part of dental papilla (SCAP) [11, 12], dental follicle (DFSCs) [13], and periodontal ligament (PDLSCs) [14, 15] (Figure 1). All these dental stem cell populations express typical MSCs markers such as Stromal-derived factor 1 (STRO-1), Melanoma-associated antigen (MUC-18) or Cd146, and Cd44 [16] but some of them also express other markers including Cd90, Cd73, Cd29 and Cd24 [11, 17]. However, the marker or combinations of markers that reliably recognize dental stem cells have not been established yet. Thus, DMSCs are often recognized by their ability to give rise to odontogenic [10, 16, 18-21], adipogenic [16, 22], chondrogenic [22], osteogenic [23], myogenic [24], and neurogenic [16, 25] lineages *in vitro* or to regenerate dental tissues *in vivo* [11, 17].

Since most of dental epithelial cells disappear shortly after tooth eruption, identifying epithelial stem cells (EpSCs) within adult dental tissues constitutes a major challenge. Putative EpSC populations have been isolated from third molars [26] and, most surprisingly, dental pulp [27]. However, to date, epithelial cell rests of Mallassez (ERM) located in the periodontal ligament (Figure 1), appear as the more promising source of EpSCs [28, 29].

Recently, pluripotent stem cells, named dental pulp pluripotent stem cells (DPPSCs) have been isolated from the dental pulp of third molars [30]. These cells show the ability to differentiate into tissues that derive from embryonic mesodermal, endodermal and ectodermal layers, suggesting their potential utility for the regeneration of both epithelial and mesenchymal dental tissues.

3. Stem cell-based dental tissue regeneration

Harmful agents (*e.g.*, caries) damage firstly the hard tissues of the tooth and then reach the dental pulp. The affected dental pulp is usually amputated (pulpotomy) or extracted (pulpectomy) and substituted with an artificial material after disinfection of the pulp cavity [3]. Although the tooth is preserved in its normal position, it is not vital anymore and it cannot fulfill completely its role [3]. Thus, the regeneration of the dentin-pulp complex represents the ideal solution and this process requires the revascularization and reinnervation of the pulp, as well as the deposition of newly generated dentin. As previously mentioned, DPSCs have the ability to differentiate into odontoblasts, endothelial cells, and neurons among other cell types. In mice, transplantation of DPSCs can regenerate both pulp and dentin tissues *in vivo* after pulpotomy [31]. DPSCs and SCAPs isolated from human third molars, seeded on a poly-D,L-lactide/glycole scaffold, and transplanted into the empty root canal space of mouse teeth, were able to refill the empty space with a newly formed vascularized pulp [17, 32]. A continuous layer of mineralized tissue resembling dentin was deposited in the existing dental

walls of the canals [17, 32]. Although these results prove that DPSCs can regenerate the dental pulp, further studies are clearly required to investigate their potential clinical applications.

Periodontitis is one of the most common infectious diseases in humans. Periodontitis is triggered by microorganisms that attach to the teeth, cause chronic inflammation and eventually destroy the periodontal tissues [3]. Studies in immunocompromized mice have shown that transplanted PDLSCs were able to regenerate the periodontium, thus indicating their huge potential for future cell-based therapies in dental clinic [3]. However, severe damage of the periodontal tissues often results in tooth loss, so it is still necessary to develop new strategies in order to potentially use entirely regenerated teeth in clinics. This could be achieved either by generating a tooth germ *in vitro* before implanting it *in vivo*, or by grafting dental stem cells in the oral cavity. In this last case, dental stem cells could be carried on tooth-shaped biomimetic scaffolds [3]. Using different scaffolds it has been possible to induce differentiation of PDLSCs and DPSCs into the various cell types composing the root and/or the periodontal tissues both *in vitro* and *in vivo* [33, 34]. Dental pulp, cementum and PDL have been obtained by transplanting subcutaneously human DPSCs that were placed into a natural scaffold composed of human dentin matrix [33]. Regarding regeneration of dental epithelium, it has been shown that ERM derived from porcine mandible can differentiate into ameloblasts after co-culture with dental pulp cells *in vitro*. These ameloblast-like cells were positive for Keratin 14 (K14) and amelogenin. Moreover, after transplantation of ERM cells combined

with primary dental pulp cells, an enamel-like tissue was produced in the implant. Histological analysis revealed that appropriate stages of amelogenesis from initiation to maturation were present in all implants. Thick enamel-dentin structures were clearly recognized, with ameloblast-like cells expressing K14 and amelogenin were found 8 weeks post-transplantation [29].

Equally important for the development of stem cell-based therapies in dentistry is the use of signaling molecules. Several molecules involved in periodontal development are already in use in the clinical practice. Long time ago, it was shown that PDGF molecules were able to stimulate periodontal healing and regeneration [35, 36]. Since then, other molecules such as BMPs [37-39] and amelogenins [40] have been used for the stimulation of periodontal tissues regeneration.

Current studies focus on the identification of the accurate population of cells, suitable signaling molecules, and desirable scaffold materials that will be used as carriers for specific cell types.

4. Safety and efficacy issues of stem cell-based therapies in dentistry

Stem cell-based therapies are both promising and challenging. The engraftment of exogenous therapeutic cells in patients must obey strict safety rules, exclude tumor formation, and avoid or minimize rejection [41, 42]. The purity, biological activity, and quantity of the injected cells should

be optimized to ensure cell functionality. Cell functionality should be tested both *in vitro* and *in vivo* in various animal models. Defined strategies should also develop to monitor the behavior and fate of the engrafted cells before any clinical trial. There is a consensus that differentiated cells that are originated from stem cells and not undifferentiated stem cells should be used directly for transplantation in the clinics [42]. Even if stem cell injection or transplantation is successful in animal models, it is important to optimize and secure stem cell-based therapeutic strategies before clinical trials.

An optimal engraftment strategy must avoid (or minimize) immune response in the host. Grafted or injected stem cells are recognized as foreign material by the immune system of the host, thus generating a cascade of events that results in the destruction and rejection of the transplanted cells. This process can compromise the immune status of the recipient. Immunosuppressive treatments that increase graft survival are not desired, since it has been shown that a correlation exists between the length of exposure to immunosuppressive drugs and the risk of malignancy after stem cell transplantation. Recent results have shown that mesenchymal stem cells from umbilical cord blood, dental pulp, periodontal ligament and bone marrow have immunosupressive properties *in vitro* [43-45]. Moreover, clonogenic nature of adult stem cells represents an advance over heterogeneous stem cells populations resulting in a more reproducible, potent immunosuppressive effect between patients [46]. Autologous cell-based therapies are advantageous because there is a minimal risk of immunological rejection and disease transmission. However, the outcome of

all tissue engineering approaches using autologous stem cell transplantation is subjective to the patient since the patient is at the same time the source and the recipient of the cells that will be used for his/her own treatment. Factors related to the age, general health status of the patient, health condition of the dental pulp and periodontium at the moment of surgery, as well as the size and site of the injury may influence the efficacy of stem cell-based dental treatments. The influence of these factors on the efficacy of cell preparations for cell-based dental treatments has not been investigated exhaustively.

The generation of iPSCs by reprogramming somatic cells via a cocktail of transcription factors [47, 48] could be advantageous towards cell-based regenerative therapies. Somatic cells have been reprogrammed and turned into pluripotent cells by the overexpression of a cocktail of 4 transcription factors (Oct4, Sox2, c-Myc and Klf4) [47]. It has been recently shown that mouse iPSCs can give rise to neural crest-like cells that can be further differentiated into odontogenic mesenchymal cells [49]. iPSCs could be generated from somatic cells of the patient, who will be donor and recipient simultaneously, thus overcoming the problems of an allogeneic immune rejection [50]. Although tempting, this potential has not been proven, since there is no yet a clear understanding of the effects that the reprogrammed cells could have to the immune system [51]. For example, studies in animal models have shown immunoreactivity toward grafted iPSCs of the same genetic background [52]. Both adult dental stem cells and induced pluripotent stem cells (iPSCs) represent an attractive source of cells for

regenerative dentistry. Nevertheless, there are still safety and immunogenicity issues that should be overcome before using them in clinics.

Although promising advances have been made in dental stem cell isolation and expansion, it is still necessary to refine these procedures. It is noteworthy that stem cells from every individual patient should be considered as specific pools and be quality controlled accordingly. It is obvious that there is no yet an ideal and unique approach for cell-based repair of dental tissues. However, rapid progress in stem cell biology and biomaterial sciences might allow the development of new methods and protocols for personalized dental treatments.

5. Nanomedicine: a giant leap forward disease diagnosis and treatment

Nanomedicine represents a subfield of nanotechnology that uses particles in the size range 1-1,000 nm for the treatment, diagnosis, monitoring, and control of various diseases [53, 54]. Nanoparticles, which are similar in scale to biological macromolecules such as DNA and proteins [53], can be used for targeted therapy through DNA, protein and drug delivery, *in vivo* imaging, diagnostics, as well as for the creation of active scaffolds and implants [55-57]. Nanoparticles can be composed of organic (*e.g.*, lipids), inorganic materials (*e.g.*, iron oxide, gold), or combinations of both types. Novel and improved nanostructured materials can be tailored by engineering their characteristics such as structure, stability, size, shape and surface properties in order to be selectively delivered to precise sites (target

regions) of the body [58]. This can be achieved through passive or active targeting mechanisms: passive targeting is enabled by the enhanced vasculature permeability during neo-angiogenesis of injured or pathological body sites, while active targeting benefits from the overexpression in the infectious or damaged areas of several cell surface molecules that can bind specifically to pre-coated nanoparticle ligands [58, 59]. Recently, a dual modular system that mimics the communication dependent recruitment of inflammatory cells to regions of disease has been developed to improve tissue target efficiency of nanoparticles [60]. Another more recent study has demonstrated the programming and assembly of DNA-based nanorobots that are able to carry molecular loads, transport chemical ingredients to target cells and stimulate their intracellular alterations [61].

These sophisticated biomaterials are increasingly being incorporated into the stem cell biology field. The combination of stem cells with innovative nanotechnology products holds great promise for applications in the biomedical arena. Fundamental challenges include stem cell expansion *in vitro* without using feeder layers, enhancement of stem cells survival after transplantation and reproducible regulation of their fate in the body [55]. The development of nanomaterials could be helpful in detecting and manipulating stem cells that will be used for tissue repair in the clinic. Nanomaterials are being used to define precisely the stem cell microenvironment through the provision of morphogenetic gradients and cell adhesion molecules, to direct stem cell fates, and to provide a template for stem cells for the formation of new tissues and organs. Furthermore,

internalization of nanoparticles, previously labeled with chelated ions, small molecules, metals and nanocrystals, by stem cells enables their detection by imaging. The physical, chemicals and biological properties of nanomaterials can be exploited to influence proliferation, attachment, fate and differentiation of stem cells [62]. This multidisciplinary approach allowed scientists to create a fully synthetic organ for transplantation after soaking a porous polymer nanocomposite tracheobronchial replica in a solution of bone marrow stem cells [63]. Although these new developments are encouraging, long-term studies are necessary before exploitation of such synthetic nanosystems in the clinics. For example, it is important to verify the non-toxicity, exclude the tumorigenic potential [64] and adverse side effects on a systemic level of nanoparticles and study their interference with the self-renewal ability of stem cells. In addition, the pharmaceutical industry has been reticent to engage with the cell-based regenerative medicine industry probably because of the complex regulatory and ethical issues [65]. This leads to uncertainty regarding the cost and time that will be required to successfully gain market approval for the nanomedicine.

5.1. Monitoring of stem cells after transplantation: magnetic nanoparticles and quantum dots

Stem cell-based regenerative therapies necessitate thorough testing firstly in animals and finally in humans. For the evaluation of the therapeutic efficacy of the transplanted stem cells it is important to track their survival, migration, fate and regenerative impact *in vivo*. Transplanted stem cells can

be assessed for long-term periods using non-invasive imaging techniques [66, 67]. Stem cells can be tracked *in vivo* after their transplantation using different strategies: initial labeling of stem cells with fluorescent dyes or magnetic nanoparticles such as the superparamagnetic iron oxide (SPIO), and stem cell transfection with several reporter genes such as the LacZ and Green Fluorescence Protein (GFP) [68, 69]. The visualization of the labeled stem cells requires either simple or complex and sophisticated imaging systems such as the magnetic resonance imaging (MRI) [66, 70, 71], computed tomography (CT) imaging [72], positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging [73]. SPIO nanoparticles (60-150 nm in diameter) are composed of biodegradable and recyclable iron and are coated with dextran or carboxydextran to prevent aggregation and ensure aqueous solubility [74, 75]. Magnetic nanoparticles can attach to the stem cell surface, but are also capable to be internalized by phagocytosis or, more often, by endocytosis [76], a process that is often facilitated by the use of coating and membrane receptor-binding agents [69, 77]. Endocytosis of magnetic nanoparticles does not affect stem cells viability, growth, fate and differentiation [78].

Quantum dots (QDs) are cadmium selenide semiconductor fluorescence-emitting nanostructures (less than 10 nm in diameter) that are used for long-term labeling of stem cells [79-81]. QDs present a number of advantages over conventional organic dyes (*e.g.*, DiI) and fluorescence proteins (*e.g.*, GFP): high brightness, superb photostability and a single excitation wavelength for multiple colors [82]. Due to their excellent optical properties,

the detection of QDs-labeled stem cells relies on imaging systems that are less sophisticated and complex than MRI. QDs have been used to monitor in real time the dynamics of various cell components [83]. Information concerning participation and clustering of multiple cell-surface molecules involved in stem cell migration and differentiation might be useful for the design of innovative scaffolds for homing stem cells before transplantation. QDs are also internalized by endocytosis, which is improved by the use of specific peptides such RGD, phospholipids, and cholera toxin [84-86]. A number of internalized QDs are transported via endosomes to the perinuclear region [87], while QDs that will not be used by the cells display an oxidative degradation [68] that could lead to mitochondria dysfunction and ultimately cell death [88]. It is possible that the composition and physical properties of QDs and magnetic nanoparticles lead to unique toxic responses [53, 85, 87]. To date there is no conclusive evidence of known human toxic responses that are exclusively caused by nanomaterials [53]. Furthermore, most of the studies demonstrated no interference of these nanoparticles in stem cell differentiation [84, 85]. However, variations in the composition, structure, size, and surface coating of nanoparticles might influence stem cell behavior and fate [88, 89].

Magnetic nanoparticles and QDs may provide valuable information about stem cell migration, anchorage, fate and differentiation in the context of dental pathology (*e.g.*, periodontitis, pulpitis, traumatic injury) and repair [3, 5, 90]. For example, internalized nanoparticles would allow monitoring kinetics and fate of the labeled stem cells that were injected into the

periodontal space or pulp chamber following dental injury (Figure 2). These approaches are necessary to evaluate the therapeutic effects of stem cells when exposed to a specific microenvironment before their application in dental clinics.

5.2. Targeting therapy: gene, protein and drug intracellular delivery

One of the most attractive concepts in manipulating the fate of stem cells, directing thus their differentiation into specific cell populations, is the use of nanomaterials for intracellular gene delivery (*e.g.*, RNAi, DNA) [91, 92]. Generally, viral (*e.g.*, adenoviruses, lentiviruses, retroviruses) and non-viral vectors (*e.g.*, lipids, polymers) can be used for cellular transfection and/or nucleofection, thus offering durable gene expression within stem cells [93-96]. Non-viral carriers have a number of advantages over viral vectors, since they exhibit low-risk immunogenicity and insertional mutagenesis, controllable toxicity, and great gene-carrying capacity [95]. Many efforts for the improvement of non-viral vectors are focused on cationic polymers that interact with negatively charged DNA or RNAi. Polymers, including poly(L-lysine)-palmitic acid, poly(L-lysine), and polyethylenimine, condense the genetic material into particles of 200-300 nm in diameter, protect them from enzymes, and facilitate cellular entrance [94, 97]. These complexes of polymers with genetic material (called “polyplexes”) have a transfection efficiency that is equivalent to the adenoviral vectors [97].

Nanoparticles, carbon nanotubes and silicon nanowire arrays have also been used for gene delivery [98, 99]. The apatite particles coated with E-cadherin and fibronectin, ensure high gene delivery capacity in stem cells [100].

Polymeric biodegradable nanoparticles of 100-300 nm in diameter could also serve as platforms to incorporate and deliver proteins and chemicals within stem cells. It has been shown that after internalization these nanoparticles accumulate in the perinuclear region and have a minimal effect on the viability and proliferation of stem cells, but a high impact on their differentiation [68].

The cytotoxicity of “polyplexes”, nanoparticles, and nanotubes has been evaluated in stem cells and the results showed that in general the toxicity correlates with the chemistry, concentration, size, shape and coating of the nanomaterials [97, 98].

5.3. Nanobiomimetics: design of bioactive scaffolds and artificial stem cell niches

The behavior of stem cells is tightly controlled by a specialized microenvironment called the “stem cell niche” [5]. Thus, this microenvironment regulates the survival, proliferation and differentiation of stem cells.

Injection of stem cells into the injured or pathological tissue limits their spreading and, in addition, does not ensure their good engraftment [101]. Injected cells could die due to the absence of trophic factors, oxygen, or lack of a suitable extracellular matrix (ECM) for their adhesion. This can be avoided by placing stem cells in biocompatible and biodegradable nanofiber scaffolds that recreate temporary the fibrous three-dimensional (3D) network of ECM and mimic the structural aspects of the stem cell niche. Hence, stem cells are anchored to the nanofibers of the scaffolds that behave as artificial stem cell niches, and then transplanted to the lesion site. This will improve stem cell survival, migration and differentiation potentials, and finally their 3D organization [101]. Stem cells cultured on nanofiber scaffolds exhibit high viability and lower mobility, and differ in morphology when compared to cells cultured on conventional substrates (*e.g.*, polystyrene) [102, 103]. Nanofibers with controlled diameter (*e.g.*, 300-1,000 nm) are composed by either natural polymers, such as collagen and silk, or synthetic polymers including poly(lactic acid) and poly(amide) [102, 104, 105]. The 3D organization, surface and chemistry of these scaffolds result in stem cell self-renewal, migration, and differentiation. Nanofiber scaffolds have high porosity and specific surface that offer an ideal environment for stem cell homing.

Identifying the appropriate stem cell populations and providing the suitable microenvironment that allows them to repair or regenerate an injured tissue is the key for a successful cell-based therapy. Nanotechnology can be used to create artificial microenvironments that will direct stem cells or progenitor

cells towards a precise fate and function. A big challenge is to engineer materials that resemble the structural complexity of stem cell niches, which represent specific anatomic locations homing stem cells and prevented them from exiting the mitotic cycle [106]. ECM molecules such as collagen, fibronectin, laminin, and proteoglycans represent the non-cellular components of the niches and are important for the creation of a particular microenvironment (*e.g.*, tooth, bone, heart). ECM provides nanoscale structures such as the 15-300 nm in diameter collagen fibrils that allow cell adhesion (via integrins) and immobilization of signaling molecules, thus influencing the fate and behavior (*i.e.*, proliferation, migration, differentiation) of stem cells [107]. The concentration, size, spacing, surface chemistry and shape (*e.g.*, ridges, grooves, pores, pits) of the artificial nanostructures (*e.g.*, nanotubes, nanolines) are important parameters for the development of cell adhesion sites that monitor stem cell behavior [108-110]. For example, it has been shown that surface irregularity (*e.g.*, nanoline grating) and diverse surface chemistries (*e.g.*, silica, poly[methyl methacrylate]) are capable to enhance adhesion, alignment, growth, and differentiation of stem cells [108, 109].

In vivo transplantation of stem cells anchored to nanofiber-based scaffolds is a technique successfully used in regenerative medicine [111, 112]. Transplanted biodegradable scaffolds act as temporary niches that guide, by controlling stem cell behavior, the formation of a new specific ECM for tissue repair. The design of tissue-specific artificial niches offers new perspectives to stem cell-based applications in dentistry for the treatment of

peculiar anatomic sites (*e.g.*, alveolar bone, dentin-pulp complex, enamel, periodontium). Furthermore, nanomaterials could be successfully used for the generation of new nanotextured “osteogenic coating” dental implants that may lead to direct bone-material contact and also bone healing in those cases in which bone is compromised. The variety of adult stem cell populations within dental tissues indicates that their differentiation potential and response to nanoscale materials may be different. However, there are not yet methodical comparative studies that will allow the assessment of nanomaterials on the various dental stem cell lines. The lack of this crucial information delays the application of stem cell-based therapies in dental clinics.

Conclusion

There is no doubt that nanotechnology offers enormous benefits and a plethora of exciting perspectives to cell-based regenerative medicines. Recent advances in nanoscale materials increase the potential to control stem cell fate, improve DNA and drug delivery, modulate the immune response to implanted cells, and create advanced scaffolds for treatment of various diseases. Nanomaterials and cell-based products must be regulated and manufactured at a low cost scale to ensure their successful application in clinics. Dentists could benefit from the use of nanoparticles to label stem cells, which after being placed on scaffolds could be further implanted into damaged dental tissues in order to regenerate them (Figure 2). The application of nanotechnology for dental purposes (nanodentistry) holds

great promise as a type of personalized medicine for the management of target-specific treatment and imaging of dental tissues.

Future perspective

Dental clinics could benefit in the near future from the combinatorial use of stem cells and nanostructures (e.*g*., creation of specific scaffolds). These devices that will contain cells could be implanted into damaged dental sites in order to regenerate them. However, there are serious issues concerning standardization of techniques, nanoparticles and stem cells that have to be solved before their clinical application in humans.

Executive summary

1. Pathology and natural regenerative potential of dental tissues

The mineralized dental tissues are vulnerable to various external harmful agents such as bacteria and acids, but also to traumatic injuries that jeopardize tooth integrity. The natural regenerative capacity of dental tissues is often insufficient to entirely restore damaged teeth.

2. Stem cell populations within dental and periodontal tissues

Dental mesenchymal stem cells (DMSCs) have been isolated from the pulp of adult and deciduous teeth (DPSCs and SHEDs respectively), apical part of dental papilla (SCAP), dental follicle (DFSCs), and periodontal ligament (PDLSCs).

Putative epithelial stem cell populations (EpSCs) have been isolated from dental pulp. However, epithelial cell rests of Mallassez (ERM) located in the periodontal ligament, appear as the more promising source of EpSCs.

3. Stem cell-based dental tissue regeneration

Studies in animals have shown that transplanted dental mesenchymal stem cells (DMSCs) were able to regenerate the periodontium and dental pulp *in vivo* thus indicating their huge potential for future cell-based therapies in dental clinics.

4. Safety and efficacy issues of stem cell-based therapies in dentistry

There are still safety and efficacy issues that need to be solved before the application of stem cell-based therapies in clinics. Immunogeneicity of the transplanted cells is one example. The potential use of iPSCs in regenerative dentistry is discussed.

5. Nanomedicine: a giant leap forward disease diagnosis and treatment

Nanomedicine represents a subfield of nanotechnology that uses particles in the size range 1-1,000 nm for the treatment, diagnosis, monitoring, and control of various diseases. Different sophisticated biomaterials are increasingly being incorporated into the stem cell biology field.

5.1. Monitoring of stem cells after transplantation: magnetic nanoparticles and quantum dots

For the evaluation of the therapeutic efficacy of the transplanted stem cells it is important to track their survival, migration, fate and regenerative impact *in vivo*. Stem cells can be tracked *in vivo* after their transplantation using different types of nanoparticles such as superparamagnetic iron oxide (SPIO) or quantum dots (QD).

5.2. Targeting therapy: gene, protein and drug intracellular delivery

Polyplexes, nanoparticles, carbon nanotubes and silicon nanowire arrays can be used for gene delivery to stem cells before they transplanting them *in vivo*.

5.3. Nanobiomimetics: design of bioactive scaffolds and artificial stem cell niches

Biocompatible and biodegradable nanofiber scaffolds constitute artificial stem cell niches that influence the survival, self-renewal and differentiation of stem cells.

Conclusion

The use of nanotechnology for dental purposes (nanodentistry) holds great promise as a type of personalized medicine for the management of target-specific treatment and imaging of dental tissues. However, there are still certain safety issues to be solved before any clinical application.

Acknowledgments

The authors wish to thank the European Science Foundation (ESF) COST Action 1005 NAMABIO, where T.A.M. is a management committee member and the representative of Switzerland. This work was supported by the Swiss National Foundation (SNSF) grants 3100A0-118332 (T.A.M.) and 31003A-135633 (T.A.M.), and funds from the University of Zurich (L.J.-R., A.W., T.A.M.).

Conflicts of interest

Conflicts of interest (personal or financial) do not exist. The authors declare that they have not received writing assistance.

Figures

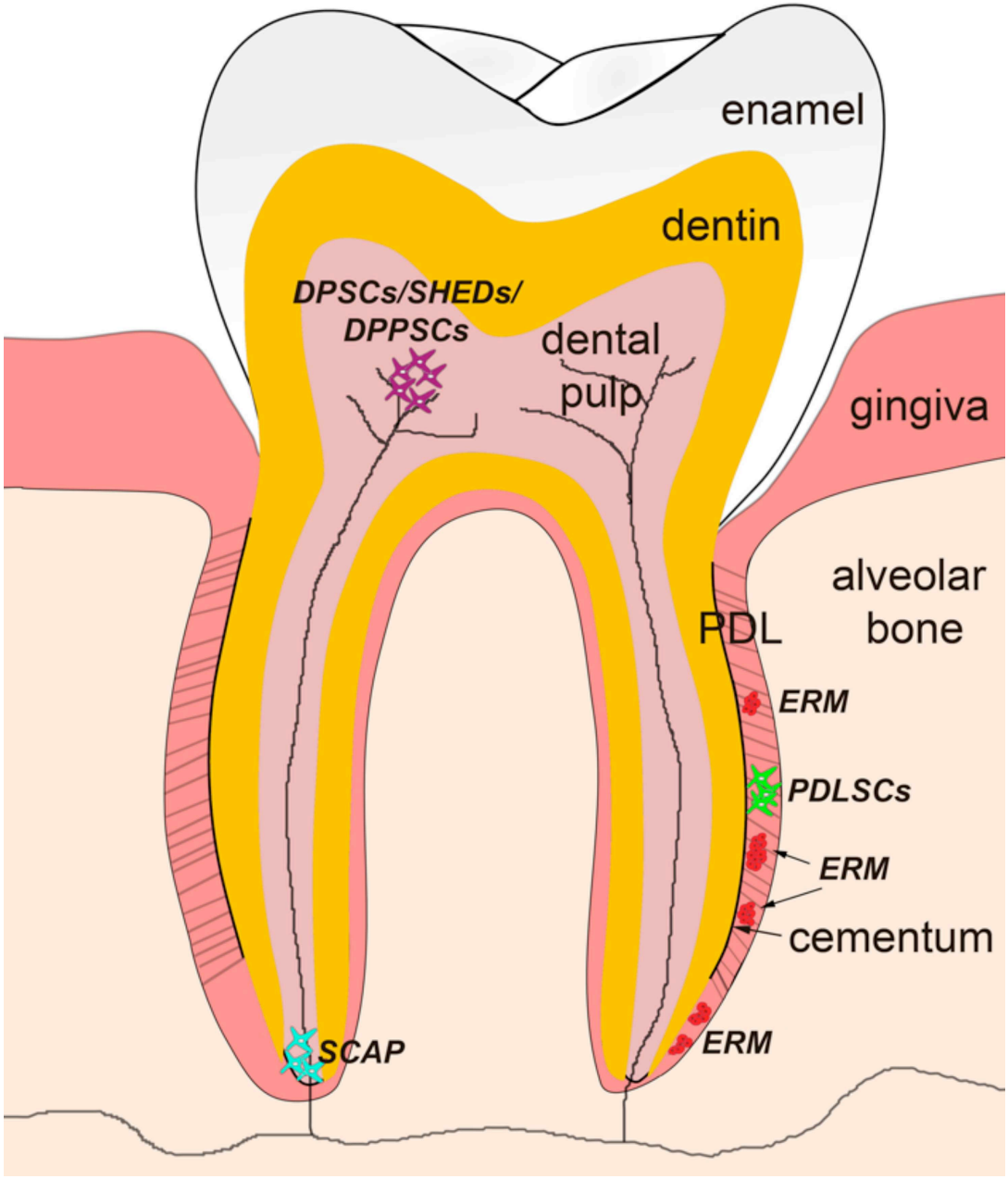

Figure 1. Schematic representation showing the various dental stem populations within an adult human tooth. Abbreviations: DPSCs, dental pulp stem cells; DPPSCs, dental pulp pluripotent stem cells; ERM, epithelial cell rests of Mallassez; PDL, periodontal ligament; PDLSCs, periodontal ligament stem cells; SCAP, stem cells from the apical papilla; SHED, stem cells from human exfoliated deciduous teeth.

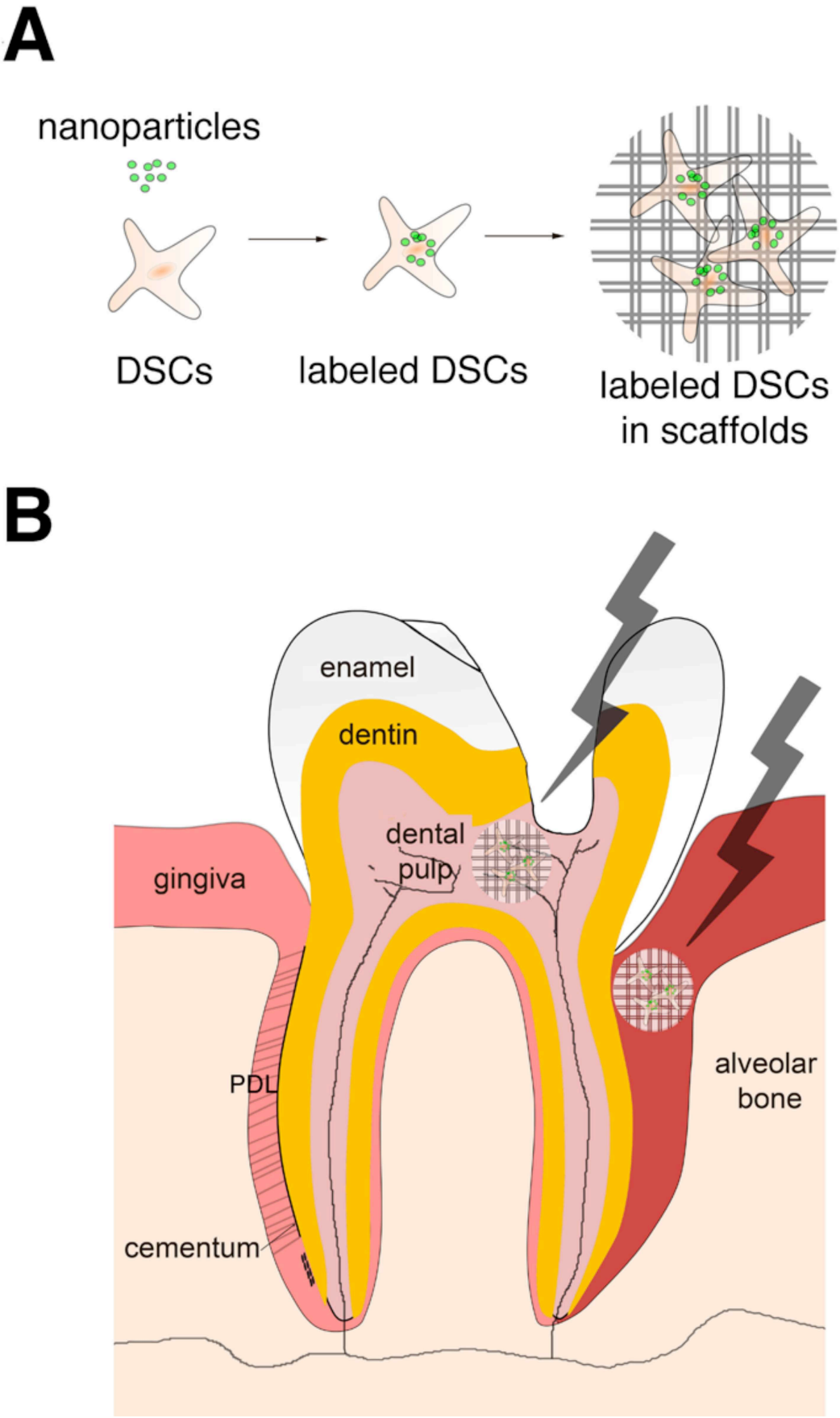
A
nanoparticles
DSCs
labeled DSCs
labeled DSCs
in scaffolds
B
enamel
dentin
dental
pulp
gingiva
PDL
cementum
alveolar
bone

Figure 2. Nanotechnology in regenerative dentistry. Dental stem cells (DSCs) can be labeled with nanoparticles before placing them into biomimetic scaffolds (A). Afterwards, those scaffolds that contain labeled DSCs could be transplanted to repair dental damaged tissues. Tooth crown, pulp and periodontium are the most commonly affected dental tissues.

References

1. Jimenez-Rojo L, Granchi Z, Graf D, Mitsiadis TA: Stem Cell Fate Determination during Development and Regeneration of Ectodermal Organs. *Frontiers in physiology* 3, 107 (2012).
2. Mitsiadis TA, De Bari C, About I: Apoptosis in developmental and repair-related human tooth remodeling: a view from the inside. *Experimental cell research* 314(4), 869-877 (2008).
3. Caton J, Bostanci N, Remboutsika E, De Bari C, Mitsiadis TA: Future dentistry: cell therapy meets tooth and periodontal repair and regeneration. *Journal of cellular and molecular medicine* 15(5), 1054-1065 (2011).
4. Mitsiadis TA, Papagerakis P: Regenerated teeth: the future of tooth replacement? *Regenerative medicine* 6(2), 135-139 (2011).**
   The main advances on dental regeration are discussed.
5. Mitsiadis TA, Feki A, Papaccio G, Caton J: Dental pulp stem cells, niches, and notch signaling in tooth injury. *Advances in dental research* 23(3), 275-279 (2011).
6. Toma JG, Akhavan M, Fernandes KJ *et al.*: Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 3(9), 778-784 (2001).
7. Zuk PA, Zhu M, Ashjian P *et al.*: Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13(12), 4279-4295 (2002).
8. De Bari C, Dell'accio F, Luyten FP: Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis and rheumatism* 44(1), 85-95 (2001).
9. Alsalameh S, Amin R, Gemba T, Lotz M: Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. *Arthritis and rheumatism* 50(5), 1522-1532 (2004).
10. Miura M, Gronthos S, Zhao M *et al.*: SHED: stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America* 100(10), 5807-5812 (2003).
11. Sonoyama W, Liu Y, Fang D *et al.*: Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PloS one* 1, e79 (2006).
12. Sonoyama W, Liu Y, Yamaza T *et al.*: Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *Journal of endodontics* 34(2), 166-171 (2008).
13. Morsczeck C, Gotz W, Schierholz J *et al.*: Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol* 24(2), 155-165 (2005).
14. Seo BM, Miura M, Gronthos S *et al.*: Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 364(9429), 149-155 (2004).
15. Wang L, Shen H, Zheng W *et al.*: Characterization of stem cells from alveolar periodontal ligament. *Tissue engineering. Part A* 17(7-8), 1015-1026 (2011).
16. Gronthos S, Brahim J, Li W *et al.*: Stem cell properties of human dental pulp stem cells. *Journal of dental research* 81(8), 531-535 (2002).
17. Huang GT, Yamaza T, Shea LD *et al.*: Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of

dentin in an in vivo model. *Tissue engineering. Part A* 16(2), 605-615 (2010).

18. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S: Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 97(25), 13625-13630 (2000). **
Dental mesenchymal stem cells are isoltaed and characterized for the first time.
19. About I, Bottero MJ, De Denato P, Camps J, Franquin JC, Mitsiadis TA: Human dentin production in vitro. *Experimental cell research* 258(1), 33-41 (2000).*
The first study showing production of human dentin in vitro.
20. Alliot-Licht B, Bluteau G, Magne D *et al.*: Dexamethasone stimulates differentiation of odontoblast-like cells in human dental pulp cultures. *Cell and tissue research* 321(3), 391-400 (2005).
21. Tecles O, Laurent P, Zygouritsas S *et al.*: Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. *Archives of oral biology* 50(2), 103-108 (2005).
22. Waddington RJ, Youde SJ, Lee CP, Sloan AJ: Isolation of distinct progenitor stem cell populations from dental pulp. *Cells, tissues, organs* 189(1-4), 268-274 (2009).
23. De Mendonca Costa A, Bueno DF, Martins MT *et al.*: Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. *J Craniofac Surg* 19(1), 204-210 (2008).
24. Kerkis I, Ambrosio CE, Kerkis A *et al.*: Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? *J Transl Med* 6, 35 (2008).
25. Nosrat IV, Widenfalk J, Olson L, Nosrat CA: Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. *Developmental biology* 238(1), 120-132 (2001).
26. Honda MJ, Shinohara Y, Hata KI, Ueda M: Subcultured odontogenic epithelial cells in combination with dental mesenchymal cells produce enamel-dentin-like complex structures. *Cell transplantation* 16(8), 833-847 (2007).
27. Nam H, Lee G: Identification of novel epithelial stem cell-like cells in human deciduous dental pulp. *Biochemical and biophysical research communications* 386(1), 135-139 (2009).
28. Nam H, Kim J, Park J *et al.*: Expression profile of the stem cell markers in human Hertwig's epithelial root sheath/Epithelial rests of Malassez cells. *Molecules and cells* 31(4), 355-360 (2011).
29. Shinmura Y, Tsuchiya S, Hata K, Honda MJ: Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. *Journal of cellular physiology* 217(3), 728-738 (2008).
30. Atari M, Gil-Recio C, Fabregat M *et al.*: Dental Pulp of the Third Molar: A New Source of Pluripotent-like Stem Cells. *Journal of cell science*, (2012).
31. Huang GT, Shagramanova K, Chan SW: Formation of odontoblast-like cells from cultured human dental pulp cells on dentin in vitro. *Journal of endodontics* 32(11), 1066-1073 (2006).

32. Volponi AA, Pang Y, Sharpe PT: Stem cell-based biological tooth repair and regeneration. *Trends in cell biology* 20(12), 715-722 (2010).
33. Washio K, Iwata T, Mizutani M *et al.*: Assessment of cell sheets derived from human periodontal ligament cells: a pre-clinical study. *Cell and tissue research* 341(3), 397-404 (2010).
34. Yang B, Chen G, Li J *et al.*: Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix - based scaffold. *Biomaterials* 33(8), 2449-2461 (2012).
35. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE: A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *Journal of periodontology* 68(12), 1186-1193 (1997).
36. Lynch SE, Williams RC, Polson AM *et al.*: A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. *Journal of clinical periodontology* 16(8), 545-548 (1989).
37. Nakashima M, Mizunuma K, Murakami T, Akamine A: Induction of dental pulp stem cell differentiation into odontoblasts by electroporation-mediated gene delivery of growth/differentiation factor 11 (Gdf11). *Gene therapy* 9(12), 814-818 (2002).
38. Jin QM, Anusaksathien O, Webb SA, Rutherford RB, Giannobile WV: Gene therapy of bone morphogenetic protein for periodontal tissue engineering. *Journal of periodontology* 74(2), 202-213 (2003).
39. Ripamonti U, Crooks J, Petit JC, Rueger DC: Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (Papio ursinus). *European journal of oral sciences* 109(4), 241-248 (2001).
40. Veis A, Tompkins K, Alvares K *et al.*: Specific amelogenin gene splice products have signaling effects on cells in culture and in implants in vivo. *The Journal of biological chemistry* 275(52), 41263-41272 (2000).
41. Grad I, Hibaoui Y, Jaconi M *et al.*: NANOG priming before full reprogramming may generate germ cell tumours. *European cells & materials* 22, 258-274; discussio 274 (2011).
42. Forsberg M, Hovatta O: Challenges for the Therapeutic use of Pluripotent Stem Derived Cells. *Frontiers in physiology* 3, 19 (2012).
43. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S: Immunomodulatory properties of human periodontal ligament stem cells. *Journal of cellular physiology* 219(3), 667-676 (2009).
44. Le Blanc K, Ringden O: Immunomodulation by mesenchymal stem cells and clinical experience. *Journal of internal medicine* 262(5), 509-525 (2007).
45. Wang M, Yang Y, Yang D *et al.*: The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Immunology* 126(2), 220-232 (2009).
46. Davies LC, Lonnies H, Locke M *et al.*: Oral mucosal progenitor cells are potently immunosuppressive in a dose-independent manner. *Stem cells and development* 21(9), 1478-1487 (2012).
47. Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663-676 (2006).**

iPSCs have been regerated for the first time.
48. Takahashi K, Okita K, Nakagawa M, Yamanaka S: Induction of pluripotent stem cells from fibroblast cultures. *Nature protocols* 2(12), 3081-3089 (2007).
49. Otsu K, Kishigami R, Oikawa-Sasaki A *et al.*: Differentiation of induced pluripotent stem cells into dental mesenchymal cells. *Stem cells and development* 21(7), 1156-1164 (2012).
50. Li SC, Zhong JF: Twisting immune responses for allogeneic stem cell therapy. *World journal of stem cells* 1(1), 30-35 (2009).
51. Okita K, Yamanaka S: Induced pluripotent stem cells: opportunities and challenges. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 366(1575), 2198-2207 (2011).
52. Zhao T, Zhang ZN, Rong Z, Xu Y: Immunogenicity of induced pluripotent stem cells. *Nature* 474(7350), 212-215 (2011).
53. Kim BY, Rutka JT, Chan WC: Nanomedicine. *The New England journal of medicine* 363(25), 2434-2443 (2010).**
The applications of nanomaterials in medicine are discussed.
54. Xia Y: Nanomaterials at work in biomedical research. *Nature materials* 7(10), 758-760 (2008).
55. Huebsch N, Mooney DJ: Inspiration and application in the evolution of biomaterials. *Nature* 462(7272), 426-432 (2009).
56. Moghimi SM, Hunter AC, Murray JC: Nanomedicine: current status and future prospects. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 19(3), 311-330 (2005).
57. Moghimi SM, Hunter AC, Andresen TL: Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective. *Annual review of pharmacology and toxicology* 52, 481-503 (2012).
58. Wang Y, Brown P, Xia Y: Nanomedicine: swarming towards the target. *Nature materials* 10(7), 482-483 (2011).
59. Petros RA, Desimone JM: Strategies in the design of nanoparticles for therapeutic applications. *Nature reviews. Drug discovery* 9(8), 615-627 (2010).**
Review of the various strategies for designing nanoparticles destined for clinical use.
60. Von Maltzahn G, Park JH, Lin KY *et al.*: Nanoparticles that communicate in vivo to amplify tumour targeting. *Nature materials* 10(7), 545-552 (2011).
61. Douglas SM, Bachelet I, Church GM: A logic-gated nanorobot for targeted transport of molecular payloads. *Science* 335(6070), 831-834 (2012).*
Elegant and smart design of nanorobots for targeted transport.
62. Oh S, Brammer KS, Li YS *et al.*: Stem cell fate dictated solely by altered nanotube dimension. *Proceedings of the National Academy of Sciences of the United States of America* 106(7), 2130-2135 (2009).
63. Jungebluth P, Alici E, Baiguera S *et al.*: Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. *Lancet* 378(9808), 1997-2004 (2011).
64. Andon FT, Fadeel B: Programmed Cell Death: Molecular Mechanisms and Implications for Safety Assessment of Nanomaterials. *Accounts of chemical research*, (2012).

65. Prescott C: Regenerative nanomedicines: an emerging investment prospective? *Journal of the Royal Society, Interface / the Royal Society* 7 Suppl 6, S783-787 (2010).
66. Arbab AS, Janic B, Haller J, Pawelczyk E, Liu W, Frank JA: In Vivo Cellular Imaging for Translational Medical Research. *Current medical imaging reviews* 5(1), 19-38 (2009).
67. Gera A, Steinberg GK, Guzman R: In vivo neural stem cell imaging: current modalities and future directions. *Regenerative medicine* 5(1), 73-86 (2010).
68. Ferreira L, Karp JM, Nobre L, Langer R: New opportunities: the use of nanotechnologies to manipulate and track stem cells. *Cell stem cell* 3(2), 136-146 (2008).
69. Thu MS, Bryant LH, Coppola T *et al.*: Self-assembling nanocomplexes by combining ferumoxytol, heparin and protamine for cell tracking by magnetic resonance imaging. *Nature medicine* 18(3), 463-467 (2012).
70. Lewin M, Carlesso N, Tung CH *et al.*: Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. *Nature biotechnology* 18(4), 410-414 (2000).
71. Terreno E, Castelli DD, Viale A, Aime S: Challenges for molecular magnetic resonance imaging. *Chemical reviews* 110(5), 3019-3042 (2010).
72. Yu SB, Watson AD: Metal-Based X-ray Contrast Media. *Chemical reviews* 99(9), 2353-2378 (1999).
73. Chen K, Conti PS: Target-specific delivery of peptide-based probes for PET imaging. *Advanced drug delivery reviews* 62(11), 1005-1022 (2010).
74. Reimer P, Balzer T: Ferucarbotran (Resovist): a new clinically approved RES-specific contrast agent for contrast-enhanced MRI of the liver: properties, clinical development, and applications. *European radiology* 13(6), 1266-1276 (2003).
75. Wang YX, Hussain SM, Krestin GP: Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. *European radiology* 11(11), 2319-2331 (2001).
76. Hsiao JK, Tai MF, Chu HH *et al.*: Magnetic nanoparticle labeling of mesenchymal stem cells without transfection agent: cellular behavior and capability of detection with clinical 1.5 T magnetic resonance at the single cell level. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 58(4), 717-724 (2007).
77. Arbab AS, Yocum GT, Kalish H *et al.*: Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI. *Blood* 104(4), 1217-1223 (2004).
78. Song YS, Ku JH: Monitoring transplanted human mesenchymal stem cells in rat and rabbit bladders using molecular magnetic resonance imaging. *Neurourology and urodynamics* 26(4), 584-593 (2007).
79. Bruchez MP: Quantum dots find their stride in single molecule tracking. *Current opinion in chemical biology* 15(6), 775-780 (2011).
80. Lin S, Xie X, Patel MR *et al.*: Quantum dot imaging for embryonic stem cells. *BMC biotechnology* 7, 67 (2007).
81. Zhang H, Yee D, Wang C: Quantum dots for cancer diagnosis and therapy: biological and clinical perspectives. *Nanomedicine (Lond)* 3(1), 83-91 (2008).

82. Alivisatos P: The use of nanocrystals in biological detection. *Nature biotechnology* 22(1), 47-52 (2004).
83. Chen H, Titushkin I, Stroscio M, Cho M: Altered membrane dynamics of quantum dot-conjugated integrins during osteogenic differentiation of human bone marrow derived progenitor cells. *Biophysical journal* 92(4), 1399-1408 (2007).
84. Chakraborty SK, Fitzpatrick JA, Phillippi JA *et al.*: Cholera toxin B conjugated quantum dots for live cell labeling. *Nano letters* 7(9), 2618-2626 (2007).
85. Shah BS, Mao JJ: Labeling of mesenchymal stem cells with bioconjugated quantum dots. *Methods Mol Biol* 680, 61-75 (2011).
86. Slotkin JR, Chakrabarti L, Dai HN *et al.*: In vivo quantum dot labeling of mammalian stem and progenitor cells. *Developmental dynamics : an official publication of the American Association of Anatomists* 236(12), 3393-3401 (2007).
87. Hsieh SC, Wang FF, Lin CS, Chen YJ, Hung SC, Wang YJ: The inhibition of osteogenesis with human bone marrow mesenchymal stem cells by CdSe/ZnS quantum dot labels. *Biomaterials* 27(8), 1656-1664 (2006).
88. Maysinger D, Lovric J, Eisenberg A, Savic R: Fate of micelles and quantum dots in cells. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* 65(3), 270-281 (2007).
89. Ruoslahti E, Bhatia SN, Sailor MJ: Targeting of drugs and nanoparticles to tumors. *The Journal of cell biology* 188(6), 759-768 (2010).
90. Mitsiadis TA, Rahiotis C: Parallels between tooth development and repair: conserved molecular mechanisms following carious and dental injury. *Journal of dental research* 83(12), 896-902 (2004).
91. Derfus AM, Chen AA, Min DH, Ruoslahti E, Bhatia SN: Targeted quantum dot conjugates for siRNA delivery. *Bioconjugate chemistry* 18(5), 1391-1396 (2007).
92. Harris TJ, Green JJ, Fung PW, Langer R, Anderson DG, Bhatia SN: Tissue-specific gene delivery via nanoparticle coating. *Biomaterials* 31(5), 998-1006 (2010).
93. Clements MO, Godfrey A, Crossley J, Wilson SJ, Takeuchi Y, Boshoff C: Lentiviral manipulation of gene expression in human adult and embryonic stem cells. *Tissue engineering* 12(7), 1741-1751 (2006).
94. Clements BA, Incani V, Kucharski C, Lavasanifar A, Ritchie B, Uludag H: A comparative evaluation of poly-L-lysine-palmitic acid and Lipofectamine 2000 for plasmid delivery to bone marrow stromal cells. *Biomaterials* 28(31), 4693-4704 (2007).
95. Glover DJ, Lipps HJ, Jans DA: Towards safe, non-viral therapeutic gene expression in humans. *Nature reviews. Genetics* 6(4), 299-310 (2005).
96. Gropp M, Reubinoff B: Lentiviral vector-mediated gene delivery into human embryonic stem cells. *Methods in enzymology* 420, 64-81 (2006).
97. Aliabadi HM, Landry B, Sun C, Tang T, Uludag H: Supramolecular assemblies in functional siRNA delivery: where do we stand? *Biomaterials* 33(8), 2546-2569 (2012).
98. Bianco A, Kostarelos K, Prato M: Making carbon nanotubes biocompatible and biodegradable. *Chem Commun (Camb)* 47(37), 10182-10188 (2011).

99. Kostarelos K: Carbon nanotubes: Fibrillar pharmacology. *Nature materials* 9(10), 793-795 (2010).
100. Kutsuzawa K, Akaike T, Chowdhury EH: The influence of the cell-adhesive proteins E-cadherin and fibronectin embedded in carbonate-apatite DNA carrier on transgene delivery and expression in a mouse embryonic stem cell line. *Biomaterials* 29(3), 370-376 (2008).
101. Mooney DJ, Vandenburgh H: Cell delivery mechanisms for tissue repair. *Cell stem cell* 2(3), 205-213 (2008).
102. Kuo SW, Lin HI, Hui-Chun Ho J *et al.*: Regulation of the fate of human mesenchymal stem cells by mechanical and stereo-topographical cues provided by silicon nanowires. *Biomaterials* 33(20), 5013-5022 (2012).
103. Shih YR, Chen CN, Tsai SW, Wang YJ, Lee OK: Growth of mesenchymal stem cells on electrospun type I collagen nanofibers. *Stem Cells* 24(11), 2391-2397 (2006).
104. Dzenis Y: Materials science. Structural nanocomposites. *Science* 319(5862), 419-420 (2008).
105. Murugan R, Ramakrishna S: Design strategies of tissue engineering scaffolds with controlled fiber orientation. *Tissue engineering* 13(8), 1845-1866 (2007).
106. Mitsiadis TA, Barrandon O, Rochat A, Barrandon Y, De Bari C: Stem cell niches in mammals. *Experimental cell research* 313(16), 3377-3385 (2007).
107. Kraehenbuehl TP, Langer R, Ferreira LS: Three-dimensional biomaterials for the study of human pluripotent stem cells. *Nature methods* 8(9), 731-736 (2011).*
The construction and benefits of the arificial stem cell niches.
108. Dalby MJ, Andar A, Nag A *et al.*: Genomic expression of mesenchymal stem cells to altered nanoscale topographies. *Journal of the Royal Society, Interface / the Royal Society* 5(26), 1055-1065 (2008).
109. Dickinson LE, Kusuma S, Gerecht S: Reconstructing the differentiation niche of embryonic stem cells using biomaterials. *Macromolecular bioscience* 11(1), 36-49 (2011).
110. Lipski AM, Pino CJ, Haselton FR, Chen IW, Shastri VP: The effect of silica nanoparticle-modified surfaces on cell morphology, cytoskeletal organization and function. *Biomaterials* 29(28), 3836-3846 (2008).
111. Hashi CK, Zhu Y, Yang GY *et al.*: Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts. *Proceedings of the National Academy of Sciences of the United States of America* 104(29), 11915-11920 (2007).
112. Hashi CK, Derugin N, Janairo RR *et al.*: Antithrombogenic modification of small-diameter microfibrous vascular grafts. *Arteriosclerosis, thrombosis, and vascular biology* 30(8), 1621-1627 (2010).